Improvements in Rapid-Mix Flow Cell Design for Sub-Millisecond Protein Folding Studies

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A small-volume mixing cell, initially designed by Dr. D. Rousseau for Raman spectroscopy and modified by our group for synchrotron IR studies has been optimized for high-pressure conditions. The optimization consists of creating a channel in a ZnSe window. It is fabricated using a custom-made brass disk (120 um thick) mounted on a rotator, and a x-z translation table that supports the ZnSe window. The table was lifted, 5 um at a time, thus touching the rotating disk, while traveling the full length (25 mm) along the x-axis of the window. Several passes of the disk along the window in the final step assured for a uniform, polished groove. The groove thickness and its depth were measured using NicPlan IR microscope, and confirmed by comparing the data obtained in the assembled flow cell with those obtained in a transmission cell with a known path-length. The cell was assembled using a flat (un-bored) ZnSe window and placed into the window holder. Initial tests show that such a cell can stand high pressures needed for the rapid-mix flow cell experiments, outstanding the "sandwich" cells having a separator (spacer) between the windows. The latter design can suffer swelling at high pressures, thus changing the solution thickness and rendering the experiment. Further optimization of the experiment accessories involves a design of a purging compartment that encompasses the flow cell and NicPlan microscope lens assembly. This eliminates the noise in the 1800 ~ 1300 cm-1 range produced by the miniature changes in the water vapor concentration during the spectral acquisition, allowing for the precise measurements and deconvolution of the Amide I stretching region. The cell covers the time region from 1 to ~20 ms, and is currently re-designed to cover both shorter (up to the dead time of the mixer, i.e. 100 us) and longer time scales (hundreds of ms).